

REMARKS

Pursuant to 37 C.F.R. 1.821-1.824, applicant submits herewith a paper copy of a Sequence Listing, a computer readable form of the Sequence Listing, and a Statement indicating that the content of the paper and computer readable copies are the same.

The Sequence Listing contains two-hundred forty-four (244) sequences. Support for the sequences in the Sequence Listing is found in the specification as follows:

Support for SEQ ID NOS: 1-58 appears in Figure 15 as filed. Support for SEQ ID NOS: 59-137 appears in Figure 16 as filed. Support for SEQ ID NOS: 138-154 appears in Figure 12 as filed. Support for SEQ ID NOS: 155 and 156 appears on pg. 46, lines 16-21 in the specification as amended. Support for SEQ ID NO: 157 appears on pg. 49, lines 13-19 in the specification as amended. Support for SEQ ID NOS: 158-195 appears in Table I on pgs. 50-51 in the specification. Support for SEQ ID NOS: 196-210 appears in Table II on pg. 52 in the specification. Support for SEQ ID NOS: 211-244 appears in Table III on pgs. 53-54 in the specification.

Applicant has amended the specification to reflect that this application is a divisional of application number 09/829,855, filed April 10, 2001, which in turn claims the

benefit of United States provisional application number 60/196,063, filed April 10, 2000 and United States provisional application number 60/196,258, filed April 11, 2000.

Applicant has also inserted after the title "This Invention was made with government support under Contract No. DE-FC36-01G011016 awarded by the Department of Energy. The Government has certain rights in this invention."

Applicant has amended pg. 12, lines 19-20 and lines 25-26; pg. 12, line 27 to pg. 13, line 1; pg. 13, lines 2-3 and lines 4-6, by substituting "Fig." with "Figure."

Applicant has further amended pg. 12, line 27 to pg. 13, line 1 by deleting "is" and replacing therefor "in" The amendment corrects an obvious typographical error.

Applicant has further amended pg. 13, lines 2-3 by deleting "is" and replacing therefor "in" The amendment corrects an obvious typographical error.

Applicant has amended the specification by adding sequence identifying numbers as required under 37 C.F.R. §§ 1.821-1.825, that is, of the SEQ ID NOS found, for example, on pg. 45, lines 8 and 24; pg. 46, lines 17 and 19-20; pg. 49, line 15, and Tables I-III. These amendments add no new matter.

Applicant has amended pg. 17, line 21 of the by deleting "at www.science.uwaterloo.ca/bpo" and substituted therefor "available through the Internet."

Amendments to the Claims

Applicant has canceled claims 1-44 without prejudice.

Applicants' cancellation of any subject matter from the claims, which is not to be interpreted as acquiescence to any outstanding rejection, is without prejudice or waiver of their right to pursue that subject matter in an application claiming priority herefrom under 35 U.S.C. § 120.


Applicant has added claims 45-56. Support for the added claims may be found throughout the specification as originally filed and as amended. For example, support for claims 45 and 46 appears on, for example, pg. 13, line 8 to pg. 14, line 8; pg. 17, lines 8-25; pg. 24, lines 10-19; pg. 25, lines 6-25; pg. 26, line 24 to pg. 27, line 17; pg. 27, line 28 to pg. 28, line 12; pg. 28, lines 15-20; pg. 28, line 24 to pg. 29, line 21; pg. 29, line 24 to pg. 30, line 5; and Examples 3 and 5 in the specification as originally filed. Support for claims 47-49 appears, for example, on pg. 28, line 24 to pg. 29, line 21 of the specification as originally filed. Support for claims 50 and 51 appears, for example, on pg. 30, line 8 to pg. 32, line 24 in the specification as

originally filed. Support for claims 52 and 53 appears, for example, on pg. 30, line 8 to pg. 31, line 25. Support for claim 54 appears, for example, on pg. 32, line 26 to pg. 34, line 19 of the specification as originally filed. Support for claim 55 appears, for example, on pg. 26, lines 8-24; and pg. 34, line 21 to pg. 35, line 16 of the specification as originally filed. Support for claim 56 appears, for example, on pg. 26, lines 8-24 of the specification as originally filed.

None of the claim amendments or additions constitutes new matter.

Applicant requests favorable consideration and early allowance of claims 45-56.

Respectfully submitted,


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APPENDIX TO SPECIFICATION AMENDMENTS

In the Specification

Page 1, underneath the title:

This application is a divisional of co-pending application number 09/829,855, filed April 10, 2001, which in turn claims the benefit of United States provisional application number 60/196,063, filed April 10, 2000 and United States provisional application number 60/196,258, filed April 11, 2000.

This Invention was made with Government support under Contract No. DE-FC36-01GO11016 awarded by the Department of Energy. The Government has certain rights in this invention.

Page 12, lines 19–20:

[Fig.] Figure 12 shows oligonucleotides useful for amplifying nucleic acid molecules for SARD.

Page 12, lines 21–24:

[Fig.] Figure 13 shows the use of the SARD strategy for Eubacteria. The double-underlined sequence and the wavy-underlined sequence represent the sequence tags for the two pools and the single-underlined sequence delineates the *Bpm*I recognition site.

Page 12, lines 25–26:

[Fig.] Figure 14 is a graphical representation of a SARD analysis of a defined population.

Page 12, line 27 to page 13, line 1:

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[Fig.] Figure 15 shows the sequence of SARD tags identified from Wy-1 sample. The number [is] in parentheses indicates the number of tags having that sequence.

Page 13, lines 2–3:

[Fig.] Figure 16 shows SARD tags identified from Wy-2 sample. The number [is] in parentheses indicates the number of tags having that sequence.

Page 13, lines 4–6:

[Fig.] Figure 17 is a graphical representation of the number and abundance of SARD tags. The upper panel shows the Wy-1 SARD Tag Diversity Profile and the lower panel shows the Wy-2 SARD Tag Diversity Profile.

Page 17, lines 7–25:

Step I. Sample Preparation and DNA Amplification by PCR

Samples may be obtained from any organism or region desired. For environmental microbial analyses, samples may be obtained from, without limitation, buildings, roadways, soil, rock, plants, animals, cell or tissue culture, organic debris, air or water. For medical microbial analyses, samples may be obtained from, without limitation, humans, animals, parasites, water, soil, air and foodstuffs. For viral analyses, samples may be obtained from, without limitation, viral culture stocks, humans, animals, plants, cell or tissue culture and microbes. For immunoglobulin or TCR analyses, samples may be obtained from, without limitation, humans, animals or cell or tissue cultures. DNA molecules from the sample of interest may be isolated by any method known in the art. See, e.g., Sambrook et al., 1989 and Ausubel et al[.], 1992. In a preferred embodiment, DNA is obtained as described by Yeates et al., “Methods for Microbiological DNA Extraction from Soil for PCR Amplification,” Biological Procedures Online, Volume 1, May 14, 1998, available through the

Internet [at www.science.uwaterloo.ca/bpo]; Liu et al., *Applied and Environmental Microbiology* (1997) 63: 4516-4522; and Tsai et al., *Applied and Environmental Microbiology* (1992) 58: 2292-2295. The DNA molecules do not have to be completely purified but only need be isolated to the point at which PCR may be performed.

Page 24, lines 8–17:

The invention is directed toward methods of analyzing the genetic diversity of a population in a sample. Each population that is analyzed will have its own unique set of different organisms or genes. The data set that is captured from each sample should recapitulate the genetic structure in a survey format to include a marker for each gene or organism and the relative abundance of each gene or organism in the population as a whole. The markers for a particular population form a marker diversity profiles (MDPs), that may be entered into a database. See, e.g., [Fig.] Figure 8 which shows one schematic for generating such a database. The method by which the data are captured is not critical as long as it produces an accurate representation of each population.

Page 25, lines 3–13:

A marker may be correlated with a particular condition or with another marker. See, e.g, [Fig.] Figure 9 for a schematic of the steps involved in determining particular parameters associated with an MDP and [Fig.] Figure 10, which shows a schematic for generating a marker diversity matrix database. A condition or state may be an environmental condition such as pH, temperature, salinity, or the presence or absence of an organic or inorganic compound such as hydrocarbons, nitrates or mineral deposits. A condition may be a physiological or medical condition such as an acute or chronic disease state, physiological state, developmental stage or associated

with a particular body tissue or fluid. Information regarding all known parameters associated with the samples will also be saved together with the MDPs.

Page 25, lines 14–22:

Each MDP is composed of markers which represent a small number, more preferably one, species or gene. For instance, in the case of Example 1, each marker would be comprised of a 12 base-pair polymorphic 16S rDNA sequence. Such parameters that relate to environmental samples may include inorganic components (obtained through atomic adsorption analysis), organic components (obtained through GC-MS or LC-MS), grain size analysis, pH, and salinity. Parameters that relate to medical samples would include, but are not limited to, a complete medical history of the donor. See, e.g., [Fig.] Figure 11, which shows a schematic for mapping applications using marker diversity profiles.

Page 45, lines 7–25:

In the Wy-1 sample, 58 distinct tags were identified and the abundance of each tag varied. The most abundant tag (ATGGCTGTCGTCAGCT) (SEQ ID NO: 6) made up about 34% of the population. This tag sequence is identical to many bacterial sequences in GenBank and its position within the 16S rDNA gene indicates that it is located in a conserved region located distal to the targeted *AluI* restriction site. In other words, the contributing 16S gene(s) for this tag did not contain the conserved *AluI* site. Since the SARD tag position is dictated by the first *AluI* site distal to the biotinylated primer used in the initial PCR reaction, it is likely that the first *AluI* site in the contributing 16S gene(s) was located downstream within a conserved region. In order to decrease the number of tags that do not contain the conserved *AluI* site next to the polymorphic region, one may gel purify the approximately 100 basepair PCR products after the first *AluI* restriction step.

However, this may result in losing some information. Nevertheless, 39% of the tags (58/148) in this set were different from each other. See Figures 15 and 17.

The Wy-2 sample was found to contain 79 different tags out of a total of 234 tags that were examined. Thus, 34% of the tags (79/234) in this set were different from each other. See Figures 16 and 17. As in the case with Wy-1, the tag ATGGCTGTCGTCAGCT (SEQ ID NO: 6), which represents a conserved sequence in a 16S rDNA gene, was most abundant and made up about 30% of the population.

Page 46, lines 16–21:

In this example, the following oligonucleotides are designed: 5' biotin-TA(CT)T(CT)CCCA(GA)GCGG(CT)(GCT)(GC)(GA)CTT(AGCT)-3' (SEQ ID NO: 155) corresponding to position 817-838 of the *Methanococcus jannaschii* 16S rDNA gene (GenBank Accession number M59126), and (5'-GGTG(TGC)CA(GC)C(CA)GCCGCGGTAA(TC)ACC(AGCT)-3' (SEQ ID NO: 156) corresponding to position 457-481 of the *Methanococcus jannaschii* 16S rDNA gene.

Page 49, lines 13–19:

An example of such a collection of degenerate oligonucleotides for the domain Bacteria could include permutations of the following primer: 5'-AACGAGCGCAACCNNNNNNNNN-3' (SEQ ID NO: 157), where N indicates any nucleotide at that position. This sequence corresponds to position 1101-1122 of the *E. coli* 16S rDNA gene (GenBank Accession number E05133). Alternatively, the primers could be designed such that they are composed of a mixture of constant sequence, semi-degenerate positions (e.g. A or G) and degenerate positions (e.g. A, G, C or T).

Page 50–54:

Table 1*

Species	GenBank Acc#	Tag Sequence	Position	SEQ ID NO:
<i>Desulfurobacterium thermolithotrophumA</i>	J001049	GTCAGTTGCCGAAGCT	814-829	158
Uncultured Aquificales OPS132	AF027104	GTCCGTGCCGTAAGCT	810-825	159
<i>Bacteroides caccae</i>	X83951	ATGGTGTGCGGACGCH	1021-1036	160
<i>Actinomyces bovis</i>	X81061	TTTCCGCGCCGTAGCT	834-849	161
<i>Actinomyces meyeri</i>	X82451	TTTCTGCGCCGTAGCT	828-843	162
<i>Denitrobacterium detoxificans</i>	AF079507	CCTCCGCGCCGCAGCT	788-803	163
Uncultured GNS bacteria BPC110	AF154084	CCCGGTAGTCCCTAGCT	765-780	164
Uncultured GNS bacteria GCA004	AF154104	CATCGGTGCCGCAGCT	824-839	165
Uncultured GNS bacteria GCA112	AF154100	CGGCGGTGCCGTAGCT	826-841	166
<i>Acetobacter aceti</i>	AF127399	ACTCAGTGTCTAGCT	782-797	167
<i>Gluconobacter asaii</i>	AB024492	ACTCAGTGTCTGAAGCT	783-798	168
<i>Burkholderia</i> sp. JB1	X92188	CCTTAGTAACGAAGCT	837-852	169
<i>Denitrobacter permanens</i>	Y12639	AGCATGTCTAAGTACGCH	789-804	170
<i>Desulfobacter curvatus</i>	M34413	CTGCTGTGCCNAAGCT	861-876	171
<i>Desulfobulbus</i> sp. BG25	U85473	CCTCTGTGTCTGCAGCT	854-869	172
<i>Legionella anisa</i>	X73394	AGCATGTCTAAGTACGCH	790-805	173
Benzene mineralizing clone SB-1	AF029039	ATGGTGTGCGGACGCH	1029-1044	174
<i>Escherichia coli</i>	E05133	CGTGGCTTCCGGAGCT	848-863	175
Uncultured Acidobacterium Sub.Div-1	X68464	CCGCCGTGCCGAAGCT	813-828	176
Uncultured Acidobacterium Sub.Div-1	Z73363	CGGCTGTGCCGAAGCT	521-536	177
Uncultured Acidobacterium Sub.Div-1	Z73365	CCACTGTGCCGTAGCT	521-536	178
Uncultured Acidobacterium Sub.Div-1	Z73368	CTGCTGTGCCGCAGCT	521-536	179
Uncultured Acidobacterium Sub.Div-1	Z73364	CTGCCGTGCCGGAGCT	521-536	180
Uncultured Acidobacterium Sub.Div-1	U68659	CCAAATGTGCCGGAGCT	319-334	181
Uncultured Acidobacterium Sub.Div-1	D26171	CCGTGCTGCCGTAGCT	79-794	182
Uncultured Acidobacterium Sub.Div-1	X97101	CCGTGCTGTCTAGCT	687-702	183
Uncultured Acidobacterium Sub.Div-1	X97098	CTGCCGTGTCTGAAGCT	798-813	184

Uncultured Acidobacterium Sub.Div-1	AF047646	CTCCCGTGTGGAAGCT	779-794	<u>185</u>
Uncultured Acidobacterium Sub.Div-1	AF050548	CCGCCGTGCCGGAGCT	316-331	<u>186</u>
Uncultured Acidobacterium Sub.Div-2	U68612	CTGAGGAACGAAAGCT	226-241	<u>187</u>
Uncultured Acidobacterium Sub.Div-2	Y07646	GTGTCGTCCCGGAGCT	830-845	<u>188</u>
Uncultured Acidobacterium Sub.Div-3	X97097	GGCTGTGCCGAAGCT	804-819	<u>189</u>
Uncultured Acidobacterium Sub.Div-3	X68466	GGTCGGTGCCGGAGCT	796-811	<u>190</u>
Uncultured Acidobacterium Sub.Div-3	X68468	GGTCGGTGCCAGAGCT	796-811	<u>191</u>
Uncultured Acidobacterium Sub.Div-3	U68648	GGTTCGTGCCGGAGCT	317-332	<u>192</u>
Uncultured Acidobacterium Sub.Div-3	X68467	TGTCTGTGCCGGAGCT	796-811	<u>193</u>
Uncultured Acidobacterium Sub.Div-3	AF013515	TATCCGTGCCGGAGCT	799-814	<u>194</u>
Uncultured Acidobacterium Sub.Div-3	AF027004	GGTCGGTGCCGGAGCT	778-793	<u>195</u>

* Sequences shown in bold with shadow indicates they are not unique to this set.

Table II

Species	GenBank Acc#	Tag Sequence	Position	SEQ ID NO:
Crenarchaeota				
<i>Aeropyrum pernix</i>	D83259	CTAGGGGGCGGGAG	614-627	196
<i>Desulfurococcus mobilis</i>	M36474	CTAGGTGTTGGGTG	856-869	197
<i>Staphylothermus marinus</i>	X99560	CTAGGTGTTGGGCG	770-783	198
<i>Metallosphaera sedula</i>	X90481	CTAGGTGTCGCGTA	756-769	199
<i>Sulfolobus acidocaldarius</i>	D14053	CTAGGTGTCGAGTA	785-798	200
<i>Sulfolobus metallicus</i>	D85519	CTAGGTGTCACGTG	744-757	201
<i>Caldivirga maquilingensis</i>	AB013926	CTAGCTGTTGGGTG	773-786	202
<i>Pyrobaculum islandicum</i>	L07511	CTAGCTGTCGGCCG	781-794	203
Euryarchaeota				
<i>Archaeoglobus fulgidus</i>	X05567	CTAGGTGTCACCGA	780-793	204
<i>Archaeoglobus veneficus</i>	Y10011	CTAGGTGTCACCGG	758-771	205
<i>Haloarcula japonica</i>	D28872	CTAGGTGTTGGCGTA	762-775	206
<i>Halococcus morrhuae</i>	D11106	CTAGGTGTTGGCGTT	765-778	207
<i>Methanococcus jannaschii</i>	M59126	CTAGGTGTCGCGTC	768-781	208
<i>Methanobacterium bryantii</i>	AF028688	None		
<i>Methanobacterium subterraneum</i>	X99045	None		
<i>Pyrococcus abyssi</i>	Z70246	CTAGGTGTCGGGCG	767-780	209
<i>Picrophilus oshimae</i>	X84901	CTAGCTGTAAATC	742-755	210

Table III

Species	GenBank Acc#	Peptide Sequence	M.W.	Position	SEQ ID NO:
<i>Desulfurobacterium thermolithotrophum</i>	AJ001049	RAQPLSLVASG*	1097.40	1079-1136	211
Uncultured Aquificales OPS132	AF027104	RAQPLSCVTSG*	1117.40	1074-1131	212
<i>Bacteroides caccae</i>	X83951	RAQPLSSVTNRSC*	1417.70	1069-1126	213
<i>Actinomyces bovis</i>	X81061	RAQPLSRVASTLWWGLAGD	2083.60	1088-1145	214
<i>Actinomyces meyeri</i>	X82451	RAQPLPYVASTLWWGLVGD	2128.60	1082-1139	215
<i>Denitrobacterium detoxificans</i>	AF079507	RAQPLPHVASIRLGTGG	1866.50	1039-1094	216
Uncultured GNS bacteria BPC110	AF154084	RAQPLLVIRVIPD	1652.10	1074-1116	217
Uncultured GNS bacteria GCA004	AF154104	RAQPSLVTRIIRD	1687.10	1080-1122	218
Uncultured GNS bacteria GCA112	AF154100	RAQPSPVIRVIRD	1669.00	1082-1124	219
<i>Acetobacter aceti</i>	AF127399	RAQPLSLVASMFGWAL*	1746.30	1038-1095	220
<i>Gluconobacter asaii</i>	AB024492	RAQPLSLVASTFRWAL*	1815.30	1034-1092	221
<i>Burkholderia</i> sp. JB1	X92188	RAQPLSLVATQHSRET	1922.20	1094-1144	222
<i>Denitrobacter permanens</i>	Y12639	RAQPLPLVATFSWAL*	1669.10	1077-1131	223
<i>Desulfobacter curvatus</i>	M34413	RAQPLSLVASTLCGNSNET	1960.40	1116-1172	224
<i>Desulfobulbus</i> sp. BG25	U85473	RAQPLPLVASSSAGHSKGT	1863.40	1114-1170	225
<i>Legionella anisa</i>	X73394	RAQPLSLVAST*	1141.40	1078-1135	226
Benzene mineralizing clone SB-1	AF029039	RAQPLPLVANRSSWGL*	1764.20	1077-1134	227
<i>Escherichia coli</i>	E05133	RAQPLSFVASGPGAGNSKET	1916.30	1103-1159	228
Uncultured Acidobacterium Sub.Div-1	Z73363	RAQPLSLVASGSSRAL*	1612.10	775-832	229
Uncultured Acidobacterium Sub.Div-1	Z73365	RAQPLSSVAIGSSRATLAK	1912.50	777-835	230
Uncultured Acidobacterium Sub.Div-1	Z73368	RAQPLFASCHH*	1933.50	779-835	231
Uncultured Acidobacterium Sub.Div-1	Z73364	RAQPLFAQLPFSFWSALCRN	2204.80	778-835	232
Uncultured Acidobacterium Sub.Div-1	U68659	RAQPLLPXAII*	1218.70	573-630	233
Uncultured Acidobacterium Sub.Div-1	D26171	RAQPLLPVATI*	1177.50	1035-1090	234
Uncultured Acidobacterium Sub.Div-1	X97101	RAQPLSPVAII*	1163.50	943-998	235
Uncultured Acidobacterium Sub.Div-1	X97098	RAQPLSSVATI*	1141.40	1054-1109	236
Uncultured Acidobacterium Sub.Div-1	AF047646	RAQPLFLVATI*	1227.60	1035-1090	237
Uncultured Acidobacterium Sub.Div-1	AF050548	RAQPSLLVANTLW*	1441.70	572-629	238

Uncultured Acidobacterium Sub.Div-2	U68612	RAQPLHVVATRKRELYVD	2150.60	577-630	<u>239</u>
Uncultured Acidobacterium Sub.Div-2	Y07646	RAQPLHVVATPQGGTLRG	1857.30	1085-1140	<u>240</u>
Uncultured Acidobacterium Sub.Div-3	X97097	RAQPSSLVANPQGHKPKGT	1972.40	1060-1116	<u>241</u>
Uncultured Acidobacterium Sub.Div-3	U68648	RARPLSCVAII*	1197.70	574-629	<u>242</u>
Uncultured Acidobacterium Sub.Div-3	AF013515	RAQPLSCVANPQGGTLRR	1969.50	1057-1112	<u>243</u>
Uncultured Acidobacterium Sub.Div-3	AF027004	RAQPSPCVATPPRAGALSGD	1950.40	1036-1096	<u>244</u>

* Indicates an in-frame stop codon was encountered within the polymorphic sequence.